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PRDM9 and its role in genetic recombination

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Abstract

PRDM9 is a zinc finger protein that binds DNA at specific locations in the genome where it trimethylates histone H3 at lysines 4 and 36 at surrounding nucleosomes. In meiosis in many species, including humans and mice where PRDM9 has been most intensely studied, these actions determine the location of recombination hotspots, where genetic recombination occurs. In addition, PRDM9 facilitates the association of hotspots with the chromosome axis, the site of the programmed DNA double strand breaks (DSBs) that give rise to genetic exchange between chromosomes. In the absence of PRDM9 DSBs are not properly repaired. Collectively, these actions determine patterns of genetic linkage and the possibilities for chromosome reorganization over successive generations.

Keywords

PRDM9; meiosis; recombination

PRDM9 FUNCTIONS IN MEIOTIC RECOMBINATION

The DNA-binding, zinc finger, histone methyltransferase PRDM9 determines the location of recombination hotspots in both male and female germ cells in a number of mammalian species. Hayashi and Matsui first described PRDM9 in mice in 2006, naming it “Meisetz”, a protein essential for meiosis but absent from somatic cells [1]. In 2009, Mihola *et al.* identified PRDM9 as the first, and still only known, mammalian hybrid sterility gene involved in speciation [2], and in 2010, three groups simultaneously identified it as the gene responsible for determining the location of recombination hotspots [3–5]. Since then, there have been multiple confirmations of PRDM9’s role in controlling human recombination hotspots [6–8], and multiple studies have reported its role in controlling hotspot locations in non-human primates [9–13], rodents [14–16], ruminants [17–21], and equids [22].

Recent years have seen considerable advances in our understanding of the molecular details of PRDM9’s interaction with DNA as well as its ability to alter the local chromatin structure by methylating histone H3K4 and H3K36. It has also become apparent that, in addition to trimethylating adjacent nucleosomes, PRDM9 associates hotspots, which originate in DNA

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loops, with the chromosomal axis, where the DNA double-strand breaks (DSBs) required for genetic exchange are formed, and assures the proper repair of DSBs.

Several reviews describe particular aspects of PRDM9 function [23–25]. Here we provide a broad overview and point out some of the open questions regarding the properties and functions of a protein that plays a key role in the mechanisms of mammalian genetic recombination and that has a major influence on the nature of genetic linkage and how patterns of chromosome reorganization can change over successive generations.

STRUCTURE OF PRDM9

PRDM9 (PR/SET domain containing protein 9) (Figure 1A) contains an N-terminal KRAB domain involved in protein-protein interactions [26, 27], an SSXRD nuclear localization signal, a SET domain providing methyltransferase activity [28–31], and a zinc finger domain containing a single, proximal zinc finger separated from the rest of the terminal C2H2 zinc finger array, which can contain anywhere from 8 to over 20 fingers [5, 7, 8, 17, 18, 32, 33]. PRDM9's protein structure is highly conserved across species except for the terminal zinc finger array, which is highly polymorphic, both in the number of fingers present and the identity of the three amino acids in each finger that contact DNA and determine PRDM9's DNA-binding specificity (Figure 1B). As discussed below, the origins of this extreme polymorphism lie in the details of the DNA repair process during genetic recombination, which continually force evolutionary selection of new *PRDM9* alleles encoding proteins with altered binding specificities.

PRDM9 FUNCTION IN MEIOSIS

Nearly all that we know about the detailed mechanisms of PRDM9 function come from studies of germline cells from juvenile male mice undergoing the first wave of meiosis; these cells are a particularly rich source of cells in the early leptotene through zygotene stages of meiosis when PRDM9 is active. Cytological assays show PRDM9 first appearing at the preleptotene to leptotene stages, when it localizes to the nucleus, and then disappearing by the end of zygotene [26, 34]. In a typical C57BL/6 germ cell, PRDM9 binds at approximately 4,700 sites, choosing from the ~16,000 sites present on each of the four sets of chromatids that arise following meiotic DNA replication [35]. Each PRDM9 variant binds a set of DNA sites characterized by a common recognition motif, which is determined by the composition of its zinc finger domain [6, 7, 32, 36]. Although PRDM9 uses all of its fingers to bind DNA, only a subset of 4–6 fingers contribute substantially to the motif [37, 38]; the remaining fingers play a secondary role in providing sequence specificity [39] and also act in a non-sequence specific manner to further stabilize binding [36]. *In vivo,* PRDM9 functions as a multimer [37, 40, 41],; a fact that has implications for hotspot choice in individuals heterozygous for PRDM9 alleles that differ in their binding affinity for DNA [40, 42]. In addition to binding DNA [4, 5], PRDM9 trimethylates histone H3 at lysine-4 [29, 35, 43] and lysine-36 [28, 30, 31, 44] at nearby nucleosomes with its SET domain, reorganizing the local chromatin structure and limiting the space in which the recombination intermediates will be resolved [35]. PRDM9 is also reported to automethylate its own lysine sequences [44]. The biological significance of this activity is as yet unknown; it would be

valuable to know whether methylated and unmethylated PRDM9 differ in their ability to bind DNA or methylate histone H3, and particularly whether automethylation affects PRDM9's stability *in vivo*, as PRDM9 disappears abruptly at the end of the zygotene stage of meiosis.

Hotspots vary over at least three orders of magnitude in their binding affinity and the likelihood that they will become trimethylated [35, 45], which influences the likelihood that a genetic crossover will eventually occur at that site [35]. Additionally, the ~16,000 sites used *in vivo* represent fewer than half of the genomic binding sites that can be detected in *in vitro* binding studies; the critical factor being the prior presence of chromatin modifications that close chromatin and prevent PRDM9 binding *in vivo* [45]. The choice between used and unused sites is independent of the relative affinity of PRDM9 for DNA at the various sites, indicating that this is not a competition between the binding energies of PRDM9 and factors that close chromatin; instead, prior chromatin modifications appear to gate whether PRDM9 binding sites can be used *in vivo* [45].

Recently, one study [46], which used a custom antibody to PRDM9, reported the presence in mice of a second, distinct class of PRDM9-containing chromosomal sites identified by ChIP-seq. These Class 2 sites do not have the characteristics of recombination hotspots as they do not contain PRDM9 DNA-binding motifs or acquire DNA double-strand breaks (DSBs). Their significance is presently uncertain as the antibody used for their detection also reacts with another, unknown protein present in *Prdm9* KO cells that are deficient in PRDM9, and Class 2 sites were not detected in other PRDM9 ChIP-seq experiments using a different anti-PRDM9 antibody [45, 47].

The nucleosomes that PRDM9 methylates become displaced laterally, creating a nucleosome depleted valley [35]. The topoisomerase SPO11 then creates DSBs within or near this valley [48]. These DSBs initiate the inter-homolog DNA exchange that gives rise to genetic recombinants [43, 48, 49]. In doing so, a homeostatic mechanism [50] mediated by the protein kinase ATM [51] assures that only ~250–400 of the ~4,700 methylated hotspots acquire a DSB. The factors determining which H3K4me3 sites acquire a DSB are as yet poorly understood.

Since hotspot locations are determined by the preferred DNA binding sites of whichever PRDM9 allele(s) are present, the initial contact of PRDM9 with DNA almost certainly occurs in the DNA loops rather than along the growing chromosomal axis. However, DNA cleavage by SPO11 [52] occurs along the chromosomal axis in concert with TOPOVIB [53], MEI4 [54] and IHO1 [55]. The necessary step of associating activated hotspots with the chromosomal axis where DSB formation occurs likely requires additional machinery, and four proteins that bind to the KRAB domain of PRDM9—EWSR1, CDYL, EHMT2 [26], and CXXC1 [26, 27]—have now been implicated. EHMT2 and CDYL are thought to bind PRDM9 early in meiosis, possibly defining the dimensions of hotspots by limiting the lateral extent of nucleosome trimethylation [26]. Later, EWSR1 complexes with PRDM9 and interacts with the meiosis-specific cohesin REC8 to associate hotspots with the chromosome axis [26]. Yeast two-hybrid data shows that CXXC1 is also able to interact with IHO1, an essential component of the meiotic DSB machinery localized on the chromosome axis [27].

It is still an open question whether additional proteins are involved or if the affinities of EWSR1 and CXXC1 for both PRDM9 and axis proteins are enough to stabilize location of hotspots on the chromosome axis.

In the widely accepted molecular model of the recombination process [56], the 5′ strand on each side of the DSB break is resected, generating single-stranded 3′ tails that invade the homologous DNA sequence on another chromatid, thus generating the Holliday Junctions that mediate the exchange of DNA between homologs. The extent of Holliday Junction migration away from the initial site of DSB formation is limited to the region of PRDM9-methylated nucleosomes, which, in turn, limits the final location of the genetic crossover that eventuates [35] (Figure 2).

In the absence of PRDM9, meiotic cells make DSBs at the remaining H3K4me3 sites, particularly those occurring in the open chromatin regions of other regulatory elements, such as gene promoters [49]. These ectopic DSBs are not repaired successfully, and cells containing them undergo pachytene arrest and apoptosis. The consequence is sterility resulting from the complete failure of both sperm and egg production. Since this failure to repair DSBs occurs in both juvenile and adult mice, it is unlikely to be related to the differences in DNA repair processes seen at the two ages [57]. This sequence of events implies that the presence of H3K4me3 alone is not sufficient to distinguish hotspots from other H3K4me3 sites and that additional factors must be involved. Obvious possibilities are the additional presence of H3K36me3, as H3K4me3 and H3K36me3 only co-occur in germ cells at hotspots [28], the additional presence of PRDM9 and its interaction with other proteins, or some combination of these factors.

EVOLUTION OF PRDM9

The repair process of the DNA sequences resected from the initiating chromatid uses the sequence of its partner as the template. Considering this directionally oriented repair, it was predicted that mutations inactivating hotspots would be under strong positive selection, with the eventual disappearance of hotspots finally resulting in loss of recombination and fertility [58]. Since contemporary species obviously reproduce, this prediction gave rise to the “hotspot paradox”, which generated much debate. The eventual discovery of PRDM9 offered a resolution of this dilemma with the realization that mutations in PRDM9’s zinc finger array can rescue sterility by creating an entirely new set of hotspots in a single step. That the evolutionary erosion of hotspots occurs and is repaired by originating new PRDM9 alleles has now been confirmed experimentally in mice [3, 47, 59, 60] and in humans [61], and is assumed to be the evolutionary pressure driving the exceptional diversity of PRDM9 alleles observed in many species.

Human populations vary considerably in their *PRDM9* allelic composition [5, 8, 32, 33], generally agreeing with their “out of Africa” origins [62]. African populations have about 50% Allele A, 13% allele C, with a wide variety of minor alleles constituting the remainder [32], while non-African populations are virtually monomorphic with ~90% alleles A and B (mostly A), which only differ in a serine/threonine substitution that does not affect binding specificity [5, 7, 8] (Figure 1B). Neanderthal and Denisovan samples contain *PRDM9* alleles

closely related to rare contemporary alleles that are limited to Africans [11, 61]. Non-human primates are highly diverse, with extensive variation in the amino acids determining DNA binding specificity [9–11].

Extensive surveys of wild mouse populations have reported well over a hundred alleles that differ in their zinc finger domains [15, 16]. Among inbred strains of laboratory mice, those derived from the *Mus musculus domesticus* subspecies largely carry either the *Dom2* or *Dom3* allele, which differ in the presence of two vs. three copies of one of the fingers [5, 63], a difference that is sufficient to create a substantial difference in binding specificity (Figure 1B). Laboratory inbred strains derived from *M.m. musculus* carry the *Msc* allele and those derived from *M.m. castaneus* the *Cst* allele (Figure 1B).

Extending earlier work on PRDM9 evolution [64–66], newer research [67, 68] used the exceptional diversity in PRDM9's zinc finger array that arises as a consequence of its role in genetic recombination to infer when PRDM9 has functioned to specify hotspot locations during its evolutionary history. All species whose PRDM9 is thought to have a function in recombination possess the canonical domain structure with intact KRAB, SSXRD, and SET domains as well as the zinc finger array. Absence of any of the domains appears to result in loss of recombination-activating function, suggesting that each domain plays an essential role. The canonical structure with high zinc finger polymorphism is present in contemporary descendants of jawless fishes (Agnathans), the most primitive vertebrates. It is also present in some bony fishes, turtles, snakes, lizards, coelacanths, and nearly all mammals that have been examined with the exception of canids. It has not been observed in amphibians, birds, and some reptiles. If recombinationally active PRDM9 was indeed present in our most primitive vertebrate ancestors, its sporadic presence in the vertebrate evolutionary tree indicates that PRDM9's function in recombination has been lost and/or gained multiple times in the course of vertebrate evolution. Primate examples of gains and losses include human *PRDM7*, a gene duplication with three amino acid substitutions in the SET domain, one of which practically destroys the ability to methylate both H3K4 and H3K36, along with loss of most of the zinc finger array [69], and tarsiers (*Tarsius syrichta),* which exhibit two frame shift mutations, one of which cancels the other to regenerate an active sequence [10]. In ruminants, multiple gene duplications have created several *PRDM9* paralogues, at least two of which appear to be recombinationally active [19, 21, 70]. In considering what the evolutionary pressures involved in these various changes might have been, the most obvious advantage to acquiring PRDM9 is sequestering DSB formation away from promoters and other functional genomic elements [49]. Less clear is what advantages might have accrued to reversing that step.

In what appears to be a remarkable case of horizontal gene transfer, the malarial parasite *Plasmodium berghei*, possesses a single zinc finger protein (PbZfp) that lacks a SET domain, but is nevertheless required for methylation of histone H3 at K4, K27 and K36, presumably by complexing with another histone methyltransferase [71]. Mice infected with parasites carrying a knockout of PbZfp survive longer than controls and show severely reduced mosquito infectivity. PbZfp-deficient parasites also have a markedly reduced ability to produce plasmodial oocysts, implicating a role in genetic recombination and the

possibility that mammalian *PRDM9* may have served as the original source of this gene [71].

PRDM9-INDEPENDENT RECOMBINATION

Although PRDM9 has been shown to play a major role in determining the location and fate of recombination hotspots in multiple mammalian species (humans, non-human primates, mice, cattle, sheep, and horses), the notable exception is canids (dogs, wolves, coyotes, and foxes), where *PRDM9* has become a pseudogene [72–74]. Despite lacking PRDM9, dogs do have hotspots, whose locations are highly stable over evolutionary time, in contrast to the rapid evolutionary erosion of hotspots in mammals with an active *PRDM9* gene [72, 74]. Canine hotspots are not associated with the locations of the H3K4me3 peaks seen in canine spermatocytes; instead they are enriched in CpG-rich regions just upstream of transcription start sites, with a preference for unmethylated CpG islands [74, 75]. These differences reinforce their lack of dependence on PRDM9. Clearly, canids rely on an alternative recombination pathway that involves recombination hotspots but does not include PRDM9-dependent mechanisms. Recent evidence suggests that an alternative pathway can also exist in a mammalian species with an active PRDM9 gene, where it can be relied upon in the absence of PRDM9 [59]. A woman has been identified who is homozygous for a null mutation in *PRDM9* and is nevertheless fertile, having borne three normal children [76].

It appears there is a fundamental difference between humans and mice in whether PRDM9 determines the location of the obligate crossover in males at the pseudoautosomal region (PAR) of the X and Y chromosomes. Evidence for both PRDM9 independent recombination at the PAR in mice [49], and PRDM9 dependent recombination at the PAR in humans [6, 77] have been presented. This difference could reflect the differences in DNA sequence – both unique and repeated – of mouse and human PARs [78].

Examining PRDM9's evolutionary history in a broader context suggests that the manner in which recombination is organized along chromosomes has undergone several major revisions over an evolutionary time scale. There is a major division between organisms that do not have hotspots and in which DSB formation follows synapsis (e.g. *Drosophila* and *C. elegans*) and organisms that do have hotspots and in which synapsis depends on prior DSB formation (e.g., budding yeast and vertebrates). Among organisms that employ hotspots but do not rely on PRDM9 (e.g., yeast [79–81], birds [82], and *Arabidopsis* [83, 84]), their hotspots tend to occur at H3K4me3 sites in open chromatin, such as promoters. Among organisms that employ both hotspots and PRDM9 to determine their locations (e.g. mammals), hotspots are more randomly located and largely sequestered away from genomic functional elements.

PRDM9 AND HYBRID STERILITY

Before PRDM9's role in recombination was recognized, one study [2] identified it as a hybrid sterility gene that can prevent gene flow between subspecies of *Mus musculus*. Meiosis in hybrid sterile male mice strongly resembles the meiotic failure seen in *Prdm9*$^{-/-}$ mutants, involving impairment of double-strand break repair, chromosome asynapsis, and

disrupted sex-body formation, with meiotic arrest at the pachytene stage. Nevertheless the two phenomena differ in at least two important aspects; PRDM9 null mutations cause meiotic failure in both sexes, whereas the sterility of *M.m. musculus x M.m. domesticus* hybrids only affects males and requires an epistatic interaction between PRDM9 and the X chromosome.

Hybrid sterility has been intensely studied in crosses between the *M.m. domesticus* C57BL/6 (B6) strain of mice carrying the $Prdm9^{Dom2}$ allele and the *M.m. musculus* PWD strain carrying the $Prdm9^{Msc}$ allele. Male B6 x PWD F1 hybrids carrying a B6 X chromosome are fertile, but reciprocal PWD x B6 F1 males carrying a PWD X chromosome are sterile [85]. As noted, the effect is sex-specific; female mice of both crosses are fertile although their meiosis still has synaptic problems [86]. The epistatic locus has been localized to the center of the X chromosome, thereby eliminating alternative explanations involving the Y chromosome, imprinted genes, or mitochondrial DNA [87]. This region, designated *Hstx2*, is a highly repetitive 4.7 Mb region of the X [88] that also has a strong effect on the overall rate of genetic recombination in fertile hybrids [89].

The role of PRDM9 in hybrid sterility is complex. Fertility is at least partially restored if sensitive F1 hybrids carry a null mutation of the *Dom2* allele, $Prdm9^{Msc/null}$, indicating that the presence of the *Dom2* allele, but not other *Prdm9* alleles, is required for meiotic arrest [42]. In contrast, fertility is also restored if the F1 hybrids carry an additional copy of *Prdm9* as a transgene. This requirement is not allele specific; rescue occurs with the *Dom3* allele [2], the human *C* allele [60], and importantly, even with the *Dom2* allele itself.

Several models have been offered to explain the molecular basis of hybrid sterility based on the presence of PRDM9 binding sites with sequence differences between the parents, causing these asymmetric hotspots to repair more slowly, increasing asynapsis, and ultimately resulting in subfertility and sterility [59, 60, 90]. As intriguing as these models are, we still lack molecular explanations for the obligatory role of the *Hstx2* region of the X chromosome, why the *Dom2* allele is so exceptional in causing hybrid sterility, and why sterility is relieved by the presence of additional copies of the *Prdm9* gene, including the *Dom2* allele.

MEDICAL IMPLICATIONS OF PRDM9 FUNCTION IN MEIOSIS

PRDM9 deficiency has been implicated in two independent reports of azoospermia in Japan [91] [92]. This contrasts with the Pakistani woman with a homozygous null mutation in *PRDM9* who was nevertheless fertile [76]. Deciding whether these differences relate to sex or reflect human variation in the presence of an alternative recombination pathway will require further data.

The first instance of a human *PRDM9 A*-allele influence on genetic diseases was reported for the frequency of human minisatellite rearrangements and chromosomal rearrangements present in Charcot-Marie-Tooth disease arising from nonallelic homologous recombination (NAHR) [8]. Subsequently, these findings were extended to 14 out of 27 recurring disease-associated chromosome rearrangement breakpoints, which coincided with locations of

human *PRDM9 A*-allele hotspots, including Charcot-Marie-Tooth disease, and Hunter and Potocki-Lupski/Smith-Magenis syndromes [6]. Many of these disease-associated breakpoints occurred within low-copy number repeat sequences, suggesting they also arose by NAHR and that presence of particular *PRDM9* alleles predisposes to particular chromosomal rearrangement disorders. There is additional evidence for the participation of PRDM9 in chromosome rearrangements at two NAHR hotspots associated with Neurofibromatosis 1 mutations [93].

European mothers of Down syndrome children resulting from nondisjunction in meiosis I combined with lack of crossovers on Chr 21 show an increased frequency of non-A *PRDM9* alleles that are predicted to have a reduced frequency of PRDM9 binding sites on Chr 21 [94]. An excess of the *PRDM9 C* allele was found in the parents of children with B-cell acute lymphoblastic leukemia [95]. This was confirmed with marginal significance in an independent study [96]. The correlation between a particular *PRDM9* allele and cancer occurs in somatic cells and the role of PRDM9 in it is not clearly understood.

CONCLUDING REMARKS

The initial discovery of PRDM9's role in the recombination process—determining the location of recombination hotspots in many mammals, including humans and mice—opened intense study of this protein's properties and functions. We now understand that during meiosis PRDM9 binds DNA at particular recognition sequences, reorganizes the local chromatin environment by methylating adjacent nucleosomes, marks hotspots for eventual DSB formation, facilitates the translocation of activated hotspots to the chromosome axis where DSB formation occurs, and is then required to assure proper repair of the DNA breaks. As a result, PRDM9 plays a key role in determining patterns of genetic segregation and linkage, as well as influencing the possibilities for chromosome reorganization over successive generations. As fruitful as these studies have been, we are now at a transition point, presented with a new generation of experimental questions and challenges (see Outstanding Questions). Solving them will likely to go beyond providing new and valuable insights into the recombination process. PRDM9 has also become a valuable model for studying the entire family of zinc finger proteins. There are over 800 genes encoding zinc finger proteins in mammalian genomes; they comprise by far the largest class of DNA binding proteins. The experimental strategies developed in the study of PRDM9 and the results obtained using them are likely to illuminate our understandings of an exceptional class of proteins engaged in myriad regulatory functions.

OUTSTANDING QUESTIONS

- PRDM9 does not act in isolation. Rather, it associates with multiple other proteins. At the least, these complexes facilitate translocation of hotspots to the chromosomal axis and participate in DSB repair. What are the identities and roles of these additional proteins in carrying out these and possibly other functions?
- What labels H3K4me3 sites as hotspots rather than as other genomic regulatory elements such as promoters?

- Are hotspots marked by:
  - the presence of PRDM9?
  - the simultaneous presence of H3K4me3 and H3K36me3 on nearby nucleosomes?
  - both?
- What is the recognition mechanism?

- Are some mammals truly polymorphic for two recombination pathways, one dependent on PRDM9 and the other not, and what is this second pathway?
  - Is this the explanation for why recombination in the X-Y pseudoautosomal region appears to be PRDM9-dependent in humans but not in mice?
- What is the molecular mechanism of PRDM9-dependent meiotic pairing and homologous chromosome synapsis?
- What is the molecular basis of PRDM9-dependent hybrid sterility, and what can the answer to this question teach us about fundamental mechanisms of recombination?
- What are the selective pressures for gain and loss of PRDM9's function in recombination over evolutionary time?
  - Are there organisms in which it participates in some aspect of recombination even when it does not specify the location of hotspots?

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TRENDS BOX

- PRDM9 influences the possibility of genetic exchange by determining the locations of meiotic recombination hotspots in most mammals.
- To do so, it uses its zinc finger array to bind specific DNA sequences.
- Pre-existing chromatin structure influences which of PRDM9's DNA binding sites are available for use *in vivo.*
- PRDM9 trimethylates histone 3 at both lysines 4 and 36, a unique activity among mammalian chromatin modifying enzymes.
  - The extent of histone 3 methylation determines the space in which recombination will occur
  - PRDM9 reorganizes local nucleosomal structure, creating a nucleosome-free center where the DNA double-strand break required for DNA exchange between chromsomes are formed.
- PRDM9 forms complexes with other proteins that associate hotspots with the chromosomal axis and affect the subsequent double-strand break initiation and repair.
- PRDM9 alleles influence human chromosomal rearrangements, causing hereditary diseases.

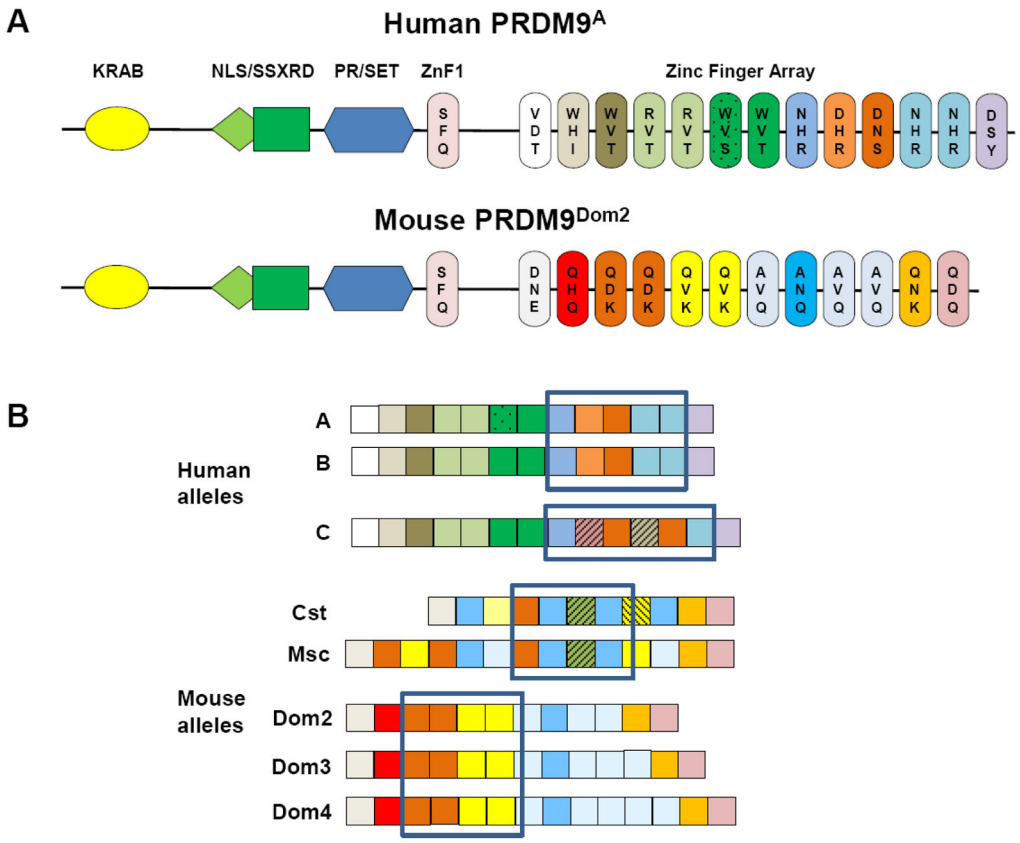

Figure 1. Molecular structure of PRDM9

A. The unique composition of the PRDM9 protein consisting of a KRAB-like, SSXRD, SET, and zinc finger array domains. The amino acid residues at -1, 3, and 6 positions relative to the α helix in each finger are responsible for the contact with DNA and are shown within the fingers. Human A and mouse Dom2 and protein variants are shown. B. Composition of the tandem zinc finger array of the major human and mouse *Prdm9/PRDM9* alleles. Individual fingers are presented as colored squares. The colors reflect differences in the amino acid residues at -1,3, and 6 positions. The boxes encompass the fingers that contribute to the principal motif of each allele [36–38, 47]. Human A allele binds a principal motif CCxCCxTxxCC with fingers 8–11 [41]. Mouse Dom2 allele binds a principal motif Gx(T/A)GxT(G/A)CT(G/A)(C/T)(C/T) with fingers 3–7 [47].

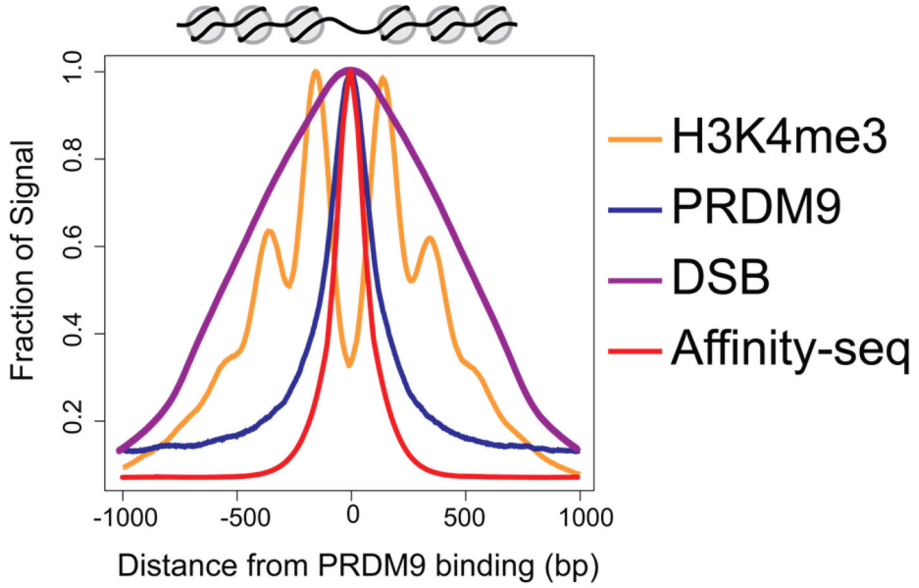

Figure 2. PRDM9 binding, nucleosome modification, repositioning, and DSB initiation

PRDM9 binding sites can be determined *in vitro* (Affinity-seq, red [45]) and *in vivo* (PRDM9 ChIP-seq, blue [47]). Its subsequent histone trimethylation at nearby nucleosomes (H3K4me3, blue; H3K36me3, orange [35]) limits the space in which double strand break initiation will occur (DMC1, purple [49]). The nucleosome repositioning after PRDM9 trimethylation of H3K4 and H3K36 is presented above the main panel.